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INTROGRESSION AND DIFFERENTIATION OF THE INVASIVE SLUG
ARION VULGARIS FROM NATIVE A. ATER

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ABSTRACT

The large arionid slug Arion vulgaris is an invasive pest dispersing through large parts of Europe and causing considerable damage in gardens, horticulture and agriculture. It is also possible that this so-called “Бепап slug” has an impact on Norwegian ecosystems, displac-ing or hybridizing with the native black slug Arion ater. The taxonomy of the large arionids is complex and confusing, encompassing different anatomical forms and colour varieties. The present study integrates, for the firsttime, coloration, ligula morphology, genital morphometry, nuclear and mitochondrial DNA in order to differentiate the large arionids found in Norway, A. vulgaris, A. ater and A. rufus. These data revealed a clear separation between A. vulgaris and A. ater based on the morphology of the genitalia and mtDNA. However, introgression with the red slug A. rufus was apparent in approximately half of the A. afer specimens analysed, evidenced by ligula morphology, mitochondrial and nuclear DNA. In addition, the mitochondrial СО! gene and the nuclear ITS1 gene suggested introgression between A. ater and A. vulgaris. Phylogenetic analyses that included A. vulgaris, A. ater and A. rufus from other parts of Europe, together with A. flagellus, A. fuscus, A. lusitanicus and A. nobrei suggest that A. vulgaris is more closely related to A. ater and A. rufus than to A. lusitanicus. This study confirms the uncertainty of identification based solely on coloration and stresses the importance of integrat-ing several approaches to differentiate these large arionids, allowing a better appreciation of their invasive potential, ecological impact and current distribution.
Key words: hybridization, mitochondrial and nuclear DNA, morphometrics, pest slug, Ari-onidae.

INTRODUCTION

Slugs are common pests in gardens, horticul-
ture as well as in agriculture, especially in cool
and moist temperate areas (Barker, 2002; Port
& Port, 1986; South, 1992). Many of these slugs are alien species, such as the grey field slug Deroceras reticulatum (Müller, 1774), which has been reported to cause extensive damage (e.g., lettuce, cabbage, grasses) (Port & Ester, 2002; South, 1992). Its inadvertent introduction and spread across Europe has led to extensive population admixture, producing a genetically diverse pest species (Foltz et al., 1984). The

invasive Arion vulgaris (Moquin- Tandon, 1855; also regarded as A. lusitanicus Mabille, 1868) has also dispersed across most of Europe, often occurring in high densities due to its high reproductive rate, catholic feeding habits and high survival rate (Kozlowski, 2007; von Pro-schwitz, 1992). It was first reported in Norway in 1988 and is currently common and locally very abundant as far north as the southern part of northern Norway (Hatteland et al., 2013a).
Considerable damage caused by A. vulgaris
has been reported in gardens, vegetable and
strawberry fields (Dolmen & Winge, 1997;
Grimm et al., 2000; von Proschwitz & Winge,

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1994). In addition, it is possible that A. vulgaris
may have a negative effect on such native
slug species as Arion ater (Linnaeus, 1758),
in Scandinavia (von Proschwitz, 1997), Austria (Reischütz, 1984a,b) and in Britain (Davies,
1987); the number of A. ater in a population falls appreciably once A. vulgaris becomes noticeable. The reason for this is unknown, but hybridization and predation have been suggested as possible explanations.
The invasive nature of A. vulgaris means there is a pressing need to distinguish this species from such native slugs as A. ater to avoid misidentifications. Unfortunately, the genus Arion poses considerable taxonomic problems, and Arion species are thus often misidentified (Noble, 1992; Quinteiro et al., 2005). This is due to intraspecific variation in morphology, colour as well as anatomical characters (Cain & Williamson, 1958; Evans, 1986; Oklans, 1922). Introgression, especially between A. ater and A. rufus (Linnaeus, 1758) (Evans, 1986), makes this situation even more confusing. However, the large Arion slugs can be differentiated using genital characters (Fig. 1); the position oft he ligula (a holdfast/stimula-tory organ for pulling the spermatophore) and the presence or absence of a lower atrium in the distal part of the genitalia (Noble, 1992). At species-level, however, the arionid group remains problematic, with a combination of morphology and genetics being mandatory to distinguish taxa and potential hybrids.
Arion ater was originally distinguished from its close relative A. rufus by colour, the former being black and the latter orange to red (van Regteren Altena, 1955). However, differences in the genitalia that corresponded to these colour variants were described by Pollonera (1889) and Quick (1947), mainly separating the two species by the bulk and relative size of the upper atrium where the ligula is situated. These taxa were even regarded as subspe-cies, hence A. ater ater and A. ater rufus (Cain & Williamson, 1958; Quick, 1960), and were distinguished by protein electrophoresis (Burnet, 1972) and multivariate statistical analyses (Noble, 1987). However, Evans (1986) found continuous variation between the two subspecies based on morphometric ratios of the distal genitalia, and Noble (1992) described intermediate variants of the ligula morphology, both authors regarding these as hybrid features. Currently, A. ater and A. rufus are considered separate species (Anderson, 2005; Falkner et al., 2001).

Arion vulgaris has traditionally been confused with A. lusitanicus, and many authors still ap-ply this name to this invasive slug taxon. Arion lusitanicus was originally thought to consist of two taxa; A. lusitanicus (sensu Mabille, 1868) and A. lusitanicus (sensu Quick = A. flagellus (Collinge, 1893; Davies, 1987)). In addition, Noble (1992) recognised two taxa (A and B) from Mont Louis in the Pyrenees of southern France that resembled A. lusitanicus based on the ligula morphology. Based on the shape of the ligula and morphometric data, Campbell (2000) added two more taxa, one sampled from the vicinity of Bergen, western Norway, and one from the Pyrenees of Spain, respectively. These were all regarded as hybrids potentially of taxa within the A. /usitanicus aggregate. Furthermore, in a molecular phylogeny, the topotype specimens (the Portuguese type localites) of A. lusitanicus and A. flagellus were grouped in an “Atlantic” clade, while other specimens of A. lusitanicus sampled outside the geographic range of the topotype were shown to be polyphyletic, and grouped together with A. ater and A. rufus (Quinteiro et al., 2005). This latter A. lusitanicus is very different from the topotype A. lusitanicus based on the form of the spermatophore and internal morphology (Castillejo, 1997), and has recently been regarded as a distinct species, A. vul-garis Moquin-Tandon (1855) (Anderson, 2005; Falkner et al., 2002). This is the first name that can be unambiguously used.
It has been proposed that A. vulgaris may hybridise with A. ater, a supposition supported by declines in the incidence of A. ater, and presence amongst the remaining individuals of intermediate colour varieties (T. Solhgy & T. von Proschwitz, pers. obs.) and genital anatomy (von Proschwitz, 2009). Observations from Germany support a similar scenario of hybridization, where the numbers of A. rufus declined after the introduction of A. vulgaris (Falkner, 1990). Mating experiments have shown that A. vulgaris and A. rufus are able to mate and subsequently lay eggs (Roth et al., 2012). Other mating studies have inves-tigated sperm exchange between these two species and showed reciprocal spermatophore exchange in mixed pairs (Dreijers et al., 2013) Anecdotal evidence exists also for negative effects of A. flagellus on A. ater in Britain, al-though it has been proposed this could derive from differences in their evolutionary tolerance of parasites and pathogens afforded by differ-ences in breeding systems (Noble 8 Jones,

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A)
Lower atrium

Upper atrium

Epiphallus

>

Vas deferens

Albumen gland
Ovotestis

Ligula

Duct of bursa copulatrix

Epiphallus

Vas deferens —\

Bursa copulatrix Y

> Conjoint duct ——

~~ Hermaphrodite duct

__— Atrium

Free oviduct

— Ligula

Albumen gland

FIG. 1. Overview of measured genital characters used for morphometrics of Arion ater (A) and A. vulgaris (B).

1996). As an obligatory outcrossing species A.
flagellus may maintain greater genetic diversity
and short-term evolutionary flexibility than the
facultative self-compatible A. ater (Noble &
Jones, 1996). Foltz et al. (1982) found a lack of genetic polymorphism in most A. afer popu-lations, thus the decline of A. ater might be a consequence of depauperate genetic variability hampering its ability to cope with the dynam-ics of host-parasite interactions (Hillebrand & Matthiessen, 2009).
The present work is an attempt to clarify the status of these large arionids with spe-cial emphasis on A. vulgaris and A. ater in Norway, by morphometric analyses, ligula anatomy, colour and molecular characterization of mtDNA and nDNA based on multiplex PCR and phylogenetic analyses. For the first time, these approaches are integrated in an attempt to determine potential introgression of exotic terrestrial slugs into the native malacofauna of Norway, affording a better understanding of their invasive potential, ecological impact and current distribution.

MATERIAL AND METHODS

Study Area

Slugs were sampled in seven sites, five of which are close to Bergen, one in Molde, northwestern Norway, and one in Klepp, south-western Norway. The areas have an oceanic climate with high precipitation throughout the year, mild winters and relatively cool summers. In addition, samples were obtained from east-ern Norway, Sweden, Denmark, U.K., France, Portugal, Germany, Iceland and Austria for molecular and phylogenetic analyses (Table 1). All slugs were dissected, and only mature specimens were used for further analyses. Mature specimens were assessed based on the presence of fully developed genitalia.

Sampling Design

All slugs were randomly handpicked and pre-liminary identification to species was based on colour and external morphology. All specimens

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TABLE 1. Arion spp. specimens included for phylogenetic analyses. Species identifications are based on genital characters including ligula.

Accession Voucher Col-
Species Specimen ID Location number number lected

Arion (Arion) ater BAH28 Керр, Rogaland, Norway JF950513 ZMBN91243 2006
(Linnaeus, 1758)
A. ater Albus200 Vestby, Akershus, Norway JF950523 ZMBN91288 2006
A. ater Wales Wales, UK JF950533 ZMBN91286 ?
A. ater SweBAH71 Torsö, Västergötland, JQ041671 ZMBN91305 2008
Sweden
Arion (Arion) rufus Germany Baden-Württemberg, JQ041672 ZMBN91304 2005
(Linnaeus, 1758) BAH7O Germany
A. rufus Denmark Silkeborg, Denmark JQ041668 ZMBN91272 2008
BAH21
A. rufus France P2 Col de la Pierre St. Martin, JF950530 ZMBN91285 1993
Pyrenees, France
A. atexr A . rufus BAHS1 Sletten, Hordaland, Norway JF950532 ZMBN91257 2010
A. atexr À . rufus England Cavedale Gorge, Derby- JF950522 ZMBN91287 ?
shire, UK
A. atexr A . rufus BA2 Керр, Rogaland, Norway JQ041673 ZMBN91239 2006
A. atexr A . rufus BA10 Керр, Rogaland, Norway JF950524 ZMBN91247 2006
A. atexr A . rufus BA23 Керр, Rogaland, Norway JF950534 ZMBN91260 2006
A. atexr A . rufus BAH49 Ha, Rogaland, Norway JF950516 ZMBN91277 2010
Arion (Arion) vulgaris BAH52 Bodg, Nordland, Norway JQ041669 ZMBN91280 2008
(Moquin-Tandon, 1855)
A. vulgaris BA16 Bergen, Hordaland, Norway JF950527 ZMBN91253 2006
A. vulgaris BA25 Bergen, Hordaland, Norway JF950529 ZMBN91266 2006
Molde, Mare og Romsdal, JF950526 ZMBN91248 2006
A. vulgaris BA11 Norway
A. vulgaris IcelandBAH72 Reykjavik, Iceland JQ041670 ZMBN91301 2004
A. vulgaris Austria Igls, Innsbruck, Austria JF950519 ZMBN91289 2
A. vulgaris AP18 Ax-les-Thermes, Pyrenees, JF950521 ZMBN91283 1992
France
A. vulgaris BP5 Mont-Louis, Pyrenees, France JF950520 ZMBN91282 1992
A. vulgaris Poland Rzeszów-Slocina, Subcar- EF520640.1 2006
pathian Region, Poland
A. vulgaris x A. ater BAH66 Odsmal, Bohuslän, Sweden JQ041665 ZMBN91303 2005
Arion (Mesarion) lus- BAH75 Arrabida, Portugal JQ041666 ZMBN91306 2010
itanicus Mabille, 1868
Arion (Mesarion) nobrei BAH77 Luso, Portugal JQ041667 ZMBN91307 2010
Pollonera, 1889
Arion (Mesarion) flagel- BAH61 Wirral, UK JF950535 ZMBN91302 7
lus Collinge, 1893
Arion (Mesarion) fuscus BAH48 Sokndal, Rogaland, Norway JF950531 ZMBN 2010
(Draparnaud, 1805)
Arion (Carinarion) silvati- 3 Bergen, Hordaland, Norway JF950517 ZMBN 2007
cus Lohmander, 1937
Arion (Carinarion) circum- 4 Bergen, Hordaland, Norway JF950518 ZMBN 2007
scriptus Johnston, 1828

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were photographed in uniform light conditions in the laboratory and killed and stored at -20°C prior to dissections following the procedure of (Evans, 1986; Noble, 1987; Campbell, 2000).

Dissections, Pictures and Morphometrics

Slugs were defrosted for approximately 20 min in warm water, placed under water and dissected in a uniform manner according to the protocol of Noble (1992).
Morphometric analysis was carried out on 54 slugs sampled in western Norway including A. vulgaris and A. ater, using 14 and 15 characters respectively. Length of organs, and for some (atria, albumen gland, ovotestis) width, was measured for all distal genitalia (Fig. 1) using an adjustable calliper in a standardised way. Some organs were pinned lightly (epiphallus and vas deferens) whilst others were mea-sured under gentle tension. Width of organs was always measured at the widest point and all measurements were repeated (up to three times) by the same person and then averaged to achieve a consistent figure. During this pro-cess, specimens were thawed and refrozen for repeated measurements at a different time point. However, freezing and thawing does not alter the shape and size of genitalia in any sig-nificant way (Evans, 1986; Noble, 1987; Camp-bell, 2000), so several cycles of this treatment were not regarded as a confounding factor. In addition, all slugs were weighed initially using Pesola® scales, on a scale graded to 15 д in intervals of 0.1 g, to obtain fresh weight. Morphometric data were analysed first by Principal Component Analysis (PCA), using the PRINC procedure in SAS/STAT (Version
6) following square root transformations. PCA was used to separate taxa without a priori as-sumptions about classification. Further analy-ses to produce the best discriminant model of the three taxa were performed using the SAS Stepdisc procedure to test the significance of each character in classification. Finally, Ca-nonical Discriminant Analysis (CDA) (Freund et al., 2003) was performed using this subset of discriminatory characters to achieve the best discriminatory model which separated A. vulgaris, A. ater and A. ater-A. rufus hybrids.

DNA Extraction, Primer Design, Multiplexes and Phylogenetics

DNA was extracted from the foot fringe, us-ing the commercially available E.Z.N.A. Tissue kit (Omega Bio-tek) and the DNeasy Blood &

Tissue kit (Qiagen) following manufacturer’s in-structions, and stored in elution buffer at 4°C. The universal invertebrate primers LCO1490 and HCO2198 designed by Folmer et al. (1994) were used to amplify the cytochrome c oxidase subunit 1 (COI) gene of A. vulgaris, A. ater and A. rufus, and primers ITS BAB18 S1F and ITS BAB28SR to amplify the internal transcribed spacer (ITS rDNA) region. Each PCR was con-ducted in 25 uL, containing 12.5 uL GoTaq PCR Mastermix from Promega® (400 uM dNTPs, 3 mM MgCl, PCR buffer), 1.0 uL of each primer (10 uM), 2.0 uL DNA (10:1 diluted) and 5.5 uL distilled H20. Amplifications were carried out in a MJ Research PTC220 Peltier thermal cycler, with cycling conditions of 94°C for 2 min, fol-lowed by 5 cycles of 92°C for 60 $, 45°C for 1.5 min, 72°C for 1.5 min, then 30 cycles of 92°C for 30 s, 50°C for 60 s and 72°C for 50 s, and a final cycle of 72°C for 7 min when amplifying the COI sequence. The ITS region, however, was amplified using cycling conditions of 94°C for 2 min, followed by 36 cycles of 92°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final cycle of 72°C for 20 min. ITS products were then cloned using the TOPO TA cloning kit from Invitrogen®, of which 24 positive colo-nies were amplified using the universal vector primers M13F and M13R in 50 uL. The cycling conditions for the latter primers were 94°C for 9 min, followed by 26 cycles of 92°C for 1 min, 55°C for 1 min and 72°C for 1.20 min, and a final cycle of 72°C for 7 min.
PCR products were checked and visualized on 1% agarose gels following standard proce-dures, before purification with ExoSap-IT (GE Healthcare) and sequenced with version 3.1 Big Dye terminator chemistry on an ABI3700 capillary sequencer (Applied Biosystems). The sequences were aligned using the software BioEdit including the multiple align-ment function of CLUSTAL W (Hall, 1999). Species-specific primers were designed for A. vulgaris, A. ater and A. rufus (Table 2) using the software PRIMER 3. The three primers for COI have been used previously to study predation by carabid beetles (Hatteland et al., 2011. 2013b).
Two diagnostic PCR multiplexes including the species-specific primers for COI and ITS were used to screen all slug specimens. Am-plifications for COI were conducted in 12.5 uL volumes, containing 6.25 uL GoTaq or Qiagen Multiplex PCR Mastermix (dNTPs, HotStarTaq DNA polymerase, 6 mM MgCl, PCR buffer), 0.25 uL of each primer (10 uM), 0.125 uL BSA (10 mg/ml), 1.0 uL DNA and 3.875 uL dH,0.

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Amplifications for ITS were also carried out in
12.5 uL volumes, containing 6.25 uL GoTag or
Qiagen Multiplex PCR Mastermix, but using
0.5 uL of each primer (10 uM), 2.0 uL ОМА (1:9
diluted) and 3.25 uL dH,0. Cycling conditions
for the COI multiplex were 94°C for 2 min, fol-lowed by 30 cycles of 92°C for 30 sec, 51°C for 1 min and 70°C for 50 sec, and a final cycle of 68°C for 5 min. The ITS multiplex was used with cycling conditions of 94°C for 2 min, fol-lowed by 35 cycles of 92°C for 30 sec, 50°C for 1 min and 72°C for 1 min, and a final cycle of 72°C for 5 min. PCR products were diluted 75:1 and analysed for absolute amplicon sizes using 7.925 uL Hi-Di formamide and 0.075 uL GeneScanTM500 Liz size standard (both Ap-plied Biosystems) and then separated using a 48-capillary 3730 DNA Analyzer (Applied Bio-systems). The software Genemapper version 4.0 (Applied Biosystems) was used to score electropherograms.
Phylogenetic analyses were performed with maximum parsimony and Bayesian methods using PAUP* version 4.0b10 (Swofford, 1998) and MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), respectively. Analyses were carried out on a sub-set of selected nucleotide sequences covering all dissimilar haplotypes from the nine species in combination with sequences depos-

ited in the NCBI GenBank database. The best fit model for the data was evaluated using the Akaike information criterion (AIC) implemented in MrModeltest version 2.2 (Nylander, 2004). А СТК + | + G model was selected with flat prior probabilites of nucleotides, invgamma distri-bution and nucleotide substitutions allowed to vary. Bayesian analysis was run using this model for 1 million generations. A consensus tree was obtained based on the Bayesian analysis with a burn in of 2000. Stationarity was determined based on a constant mean and variance. A consensus tree was also obtained with the parsimony criterion by using a heuristic search adding the sequences randomly and using the tree-bisection-reconnection (TBR) branch-swapping algorithm. Bootstrapping (Felsenstein, 1985) with 500 replicates was used to obtain confidence of nodes.

RESULTS

A total of 26 A. ater and 28 A. vulgaris specimens were dissected and scored for
morphometric data (including ligula), weight,
coloration, NDNA and mtDNA (Appendix 1 &
2). In addition, 20 A. vulgaris, 13 A. ater, 15
A. rufus, 1 A. flagellus, 2 A. lusitanicus, 2 A.
nobrei, 2 A. fuscus, 1 A. silvaticus, 1 A. circum-

TABLE 2. Primers (5’-3’) designed from the COI sequence of the mtDNA (top five primers) and ITS sequence of nrDNA (bottom two primers) to detect Arion vulgaris, A. ater and A. rufus, including size of amplified fragment.

Fragment size
Species Primer name Primer sequence | (bp)

A. vulgaris A.|.-Co1-F 1 GCCCCCATCTTTACTTTTACTTATTTGCTCC 310
GTATGGTAATAGCCCCCGCCAATACG
A. vulgaris A.l.-Co1-R2 FAM-CACCACTGAGAGGAGCC
GCTCCAGCCAATACAGGTAAAG
A. ater A.a.-Co1-F-new MTTACTTATCGGTGCGC 225
GCTCCAGCCAATACAGGTAAAG
A. rufus A.r.-Co1-F1 GCCCCCATCTTTACTTTTACTTATTTGCTCC 362
GTATGGTAATAGCCCCCGCCAATACG
A. ater and A.a.-Co1-R1 FAM-CACCACTGAGAGGAGCC
A. rufus GCTCCAGCCAATACAGGTAAAG
A. rufus, A. rufus-F AGGATCATTATCGGTCTCG 547
A. ater and 537
A. vulgaris 544
А. ater, aterand A. ater-R PET-GGGCAGGAAGAGAAGAACAA
A. vulgaris

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scriptus as well as 10 possible hybrids between determined as hybrids based on a large and A. ater and A. vulgaris were included in the highly variable ligula in the upper atrium which genetic analyses. These latter specimens were deviated from A. ater in both size and shape.

FIG. 2. Ligula types found in Arion ateri n Norway. A: Typical ligula of Arion ater, B-F: Variation in ligula of A. ater possibly introgressed with A. rufus. Specimens A, B, C, D, E and F corresponds with the specimen codes 109, 22S, 23S, 80S, 77S and 96S in Appendix 1, respectively. Pictures taken using an Olympus DP10 camera connected to a stereomicroscope with 4.8 times magnification.

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FIG. 3. Ligula types of Arion vulgaris in western Norway. A: Typical type (correspond to 67S in Appen-dix 2); B: Non-typical ligula of A. vulgaris (correspond to 12F in Appendix 2). Pictures taken using an Olympus DP10 camera connected to a stereomicroscope with 4.8 times magnification.

It also differed from A. rufus having a ligula
with several folds and not forming the fleshy
collar typical of A. rufus. All of these assumed
hybrids were sampled in mixed populations of
A. vulgaris and A. ater. Specimens representing
different haplotypes based on COI sequences (Table 1) were submitted to GenBank (acces-sion numbers JF950511-JF950535). Coloration of A. ater and A. vulgaris was similar in Some specimens, confirming earlier studies (Quick, 1960), and making it impos-sible to assign dark brown specimens to a taxon solely on coloration. Even almost black specimens of A. vulgaris were found (2 out of 28 of the specimens scored for morphometrics),

TABLE 3. Principal Component (PCA) and Ca-nonical Discriminant Analysis (CDA) components of morphometrics of the distal genitalia of Arion vulgaris and A. ater.

Eigen- Per- Cum Canoni-
Analysis Axis value cent Percent cal Corr

PCA РОТ 5.5153 58.239. 68.233 = РС? “1,8396 22.761: 91000 - -

PES 0.3832 4.742 95.742 =

CDA-A CD1 3.5768 95.376 95.376 0.8840 CD2 0.1734 4.624 100.000 0.3844

CDA-B CD1 11.6820 96.437 96.437 0.9598 CD2 0.2746 2.267 98.704 0.4641

leaving only red to brown varieties as reliable markers of identity.
The morphology of the genitalia (including ligula) proved to be distinctive in both A. vul-garis and A. ater (Figs. 1-3), confirmed through morphometric analyses (Figs. 4, 5). The ligula gave the free oviduct of the former a bulky ap-pearance, but is situated in the upper atrium of A. ater, where the epiphallus and bursa copulatrix insert (Fig. 1). Moreover, the ligula of A. vulgaris was considerably larger than that of A. afer. All A. vulgaris specimens in this study were identified as A. vulgaris according to the shape of the ligula (Fig. 3A), except for a peculiar ligula in one specimen (Fig. 3B). The ligula ofA . vulgaris is similar to A. rufus, being C-shaped and asymmetrical (Noble, 1992), although the C-shape is less pronounced and both ridges are much longer and thinner than in A. rufus. The ligulae of A. lusitanicus taxa (т-cluding Mabille, 1868) are quite distinct, being much more symmetrical (Noble, 1992).
The ligula of A. ateris symmetrical and poorly ornamented, forming a “bowl”, rather than an asymmetrical “C” as found in A. rufus (see No-ble, 1992). However, approximately 50% of the A. ater specimens deviated from this charac-teristic shape, possibly indicating introgression with A. rufus. Possible introgressed specimens were evident in all except the Molde population. A total of six different variants were found, each strikingly different with a consistent ligula shape as also found by Noble (1992) (Fig. 2).
The first two principal components (PCs) (describing 91% of the total variance) clearly

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PC1 (68.2%)

FIG. 4. Principal Component Analysis (PCA) ordination plot of morphometrics of the distal genitalia of Arion vulgaris and A. ater, blue dots — A. vulgaris; green dots — A. ater (with A. aterligula & A. ater DNA); green crosses — A. ater with A. rufus DNA but A. ater ligula; red crosses — A. ater -A . rufus hybrids as defined by ligula. First two axes represent 91% of total variance (PC1 = 68%; PC2 = 23%).

separated A. vulgaris from A. ater based on the
morphometrics (Table 3, Fig. 4). Eigenvalues
of the first principal component (68% of the
total variance) suggest it may be interpreted
primarily as a vector describing lengths of the
vas deferens, epiphallus and free oviduct (Table 4), with the lengths of the upper atrium and ligula showing lower but still positive loadings. The second principal component (nearly 23% of the total variance) showed a strong positive loading for lengths of the bursa copulatrix and epiphallus (Table 3), with lower but negative loadings for lengths of the vas deferens, free oviduct and upper atrium. Arion vulgaris was clearly differentiated from A. ater by the second PC, suggesting this species has a relatively longer epiphallus and bursa copulatrix, but shorter vas deference, free oviduct and upper atrium; confirming morphological observations (Noble, 1992). Two clusters are apparent on the first PC, each a mix ofA . afer and possible A. ater-A. rufus hybrids. However, those indi-viduals with a relatively higher PC1 score are predominately from Klepp, a locality where in-trogression seem still apparent. Two specimens from Sletten have a possible A. ater-A. rufus hybrid ligula, but A. vulgaris mtDNA as well as PC2 score consistent with A. vulgaris.

Stepwise analysis suggested four significant discriminant characters separate A. ater and A. vulgaris (Table 5). These four characters (lengths of the epiphallus, bursa copulatrix, vas deferens, and upper atrium) were used in a canonical discriminant analysis to produce a strong discriminatory model which was 87.5%

TABLE 4. Eigenvectors of genitalia characters in the Principal Component Analysis (PCA) of morphometrics of the distal genitalia of Arion vulgaris and A. ater.

Genitalia characters PC1 PC2 PCS

Epiphallus length 0.50408 0.25999 -0.75021 Vas deferens length 0.52993 -0.26874 0.44116 Free oviduct length 0.49839 -0.17338 0.15417 Ligula length 0.23898 0.04436 0.08206 Upper atrium length 0.28275 -0.17965 0.11220 Upper atrium width 0.16283 0.07457 -0.12417 Bursa copulatrix 0.19671 0.10047 -0.07286 duct length
Bursa copulatrix
length

0.12007 0.88330 0.42277

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TABLE 5. The four significant genitalia characters from the Stepwise analysis.

Genitalia characters Estimate nDF Wald/Score ChiSq “Sig Prob”

Epiphallus length -1.18006
Vas deferens length 1.74887
Upper atrium length 2.86354
Bursa copulatrix length -2.33026

accurate (Wilk’s test p < 0.001). Morphological observation suggests that the longer upper atrium of the A. ater-A. rufus complex is not an unexpected discriminatory character, but the greater length of the bursa copulatrix in A. vulgaris has not been noted before. Whilst A. vulgaris is clearly differentiated there was no

Canonical2

-6 -5 -4 -3 -2 «1 Canonicall

1 3.03014 0.0817
1 7.35874 0.0067
1 3.85600 0.0496
1 4.64053 0.0312

unambiguous clustering of A. afteorr the various assumed À. ater x A. rufus hybrid morpholo-gies; A. aterspecimens are situated atone end
of the spectrum, while the assumed hybrids are
much more dispersed (Fig. 5).
A413-bp sequence of the COI gene (mtDNA)
was obtained for 29 specimens representing 9

0 1 2 3 4 5

FIG. 5. Canonical Discriminant Analysis (CDA) ordination plot of morphometrics of the distal genitalia of Arion ater, A. ater x A. rufus and A. vulgaris. Circles represent 50% contours, ¡.e. the area in which 50% of the data lies. First two axes represent 100% of variance, producing a discriminatory model which is 87.5%; | epi 2 = length of epiphallus, | bc 2 = length of bursa copulatrix, | ua 2 = length of up-per atrium, | vd 2 = length of vas deferens. Hybrids are defined according to morphological and genetic characters.

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Arion species; A. vulgaris, A, ater, A. rufus, A. flagellus, A. lusitanicus, A. nobrei, A. fuscus and the outgroup species A. silvaticus and A. circumscriptus. The transition/transversion ratio was 2.31 while an overall bias in the nucleotide frequencies existed towards T, and mean frequencies (A = 0.28, C = 0.16, G = 0.16, 7 = 0.40) showed homogeneity among taxa (x2 = 19.51, df = 84, P = 1.00). The ob-served sequence divergence ranged from 10.5-13.8% between A. ater, A. vulgaris and A. rufus, whereas variation within species was low (0-1.3%) when restricting comparisons within Scandinavia and central Europe (Ger-many, Poland and Austria). Previous studies in Poland have shown slightly larger intraspecific variation in what was reported as invasive A. lusitanicus (Soroka & Kaluski, 2011). Interest-ingly, the single A. vulgaris specimen sampled in Austria was identical to two of the A. vul-garis specimens sampled in Norway, while the Pyrenean A. vulgaris (also regarded as A. lusitanicus A and B (Noble, 1992)) diverged from Norwegian A. vulgaris in the range of 1.2-3.4%. Furthermore, the Iberian taxa (A. lusitanicus and A. nobrei) diverged from the widespread taxa (A. vulgaris, A. ater and A. rufus) in the range of 16.9-18.6%, while A. flagellus diverged similarly from the widespread species (16.2—18.9%) and slightly less from the Iberian taxa (15.5-15.7%). Arion ater showed identical COl-sequences comparing Norwegian and Swedish specimens, while A. ater sampled in Wales diverged greatly (11.3%) from the Scandinavian specimens. Arion rufus showed also high variability when compared with specimens from the UK as well as Pyrenean specimens (0-8.7%).
The suggested introgression between A. ater and A. rufus was confirmed by the COl and ITS multiplexes, showing species-specific СО! and ITS profiles of A. rufus respectively in 27% and 23% of the specimens, determined as A. aterand A. ater x A. rufus hybrids based on morphology (Appendix 1). Most of these specimens were sampled from the southwest of Norway (Klepp and Ha populations), but also some from a site in Bergen. No diagnostic pattern was found for potential hybrids, neither for coloration nor ligula shape, although there was a tendency for unusual ligula variants to be coupled with molecular markers suggesting introgression (Appendix 1). Furthermore, A. vulgaris COl-sequences were amplified in two specimens with A. ater morphology sampled from the same site; Sletten (Bergen), of which

one specimen also showed ITS-sequences of A. vulgaris. We also amplified ITS-sequences ofA . vulgaris in one additional specimen iden-tified as A. ater as well as ITS-sequences of A. ater. Both of these specimens had a ligula anatomy suggesting introgression from A. rufus as well as ITS-sequences ofA . rufus, their col-oration was black. All the A. vulgaris individuals showed concordance of mtDNA and morphol-ogy (Appendix 2). The same concordance was also found between nDNA and morphology in A. vulgaris, except for one specimen with an A. ater |TS sequence, one with both A. vulgaris and A. ater ITS profiles and one specimen with A. vulgaris and A. rufus ITS profiles. Phylogenetic analyses of the COl-sequences, using the parsimony criterion supported the morphological results, indicating A. ater and A. rufus grouped together more closely than with A. vulgaris (Fig. 6). This finding as well as most of the other topologies was supported by high bootstrapping values. However, the Bayesian analysis suggested that A. afer is more closely related to A. vulgaris, although this showed a low node support (Fig. 7). In general, the consensus tree based on the Bayesian analysis had low support in all the basal topologies. However, the Iberian taxa, A. lusitanicus and A. nobrei, was separated from the widespread species A. vulgaris (or non-topotype A. lusitanicus), A. ater and A. rufus in both phylogenies. The mtDNA data of the widespread species did not support the morphological identifications for all specimens, which is probably due to introgression. The sug-gested hybrids between A. vulgaris and A. ater varied in mtDNA haplotype, most possessing A. vulgaris sequences, although some had A. rufus haplotypes (Figs. 6, 7).

DISCUSSION

Our results confirm and emphasise dif-ferences between Arion vulgaris and the A. ater aggregate morphologically, although the mitochondrial and nuclear data suggest some introgression between A. vulgaris and A. ater. Differences between taxa and the existence of hybrids are strongly supported by morpho-metric data (Figs. 4, 5) and illustrated by the position and form of the ligula (Figs. 1-3). Mitochondrial DNA-sequences show a closer relationship among A. vulgaris, A. ater and A. rufus compared to Iberian taxa such as the topotype A. /usitanicus (Figs. 6, 7).

314 HATTELAND ET AL. A.silvaticus
A.circumscriptus
A.fuscusBAH47
A.flagellusBAH61
A.aterxA.rufusBAH49

Bar ArufusDenmark

A.rufusGermanyBAH70
a A.vulgarisxA.aterBAH66
A.aterxA.rufusBA10
77 85
A.aterxA.rufusBA2
A.vulgarisicelandBAH72
A.rufusFrance

100

100

lag A.aterWales

A.aterxA.rufusEngland
A.aterAlbus200
100
98 A.aterBA8
62 A.aterSweBAH71
A.vulgarisB
100 A.vulgarisA
A.aterxA.rufusBA23

85
90

Avu lgarisBA25

A.aterxA.rufusBAH51
62

A.lusitanicusPortugalBAH75
100
A.nobreiBAH77

FIG. 6. Phylogenetic consensus tree of COl-sequences of large Arion species in Europe using the parsimony criterion, with 500 bootstrapping replicates for node support. Tree length = 357, Consistency index (Cl) = 0.6583, Homoplasy index (HI) = 0.3417, Reten-tion index (RI) = 0.8544. Rescaled consistency index (RC) = 0.5624. Species and hybrid names are given based on genital morphology including ligula; green = A. vulgaris, blue = A. ater, red = A. rufus.

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A.silvaticus
1.00
A.circumscriptus

A.fuscusBAH47

A.flagellusBAH61

A.lusitanicusPortugalBAH75
0.99
A.nobreiBAH77

A.vulgarisB
1.00
A.vulgarisA

A.vulgarisB1A1
0.85
A.aterxA.rufusBAH51
0.90) |4 0.34
A.vulgarisBAH52

Te ee
| Ort
0.53 + A.vulgarisPoland

0.51

0.38

0.99 A.aterAlbus200

A.atedW ales

1.00
A.vulgarisicelandBAH72
0.27
A.rufusFrance
0.22
0.82 A.aterxA.rufusEngland

A.aterxA.rufusBAH49
1.00
A.vulgarisxA.aterBAH66

0.29
A.aterxA.rufusBA 10
1.00
0.1 A.aterxA.rufusBA2

FIG. 7. Phylogenetic consensus tree of COl-sequences of large Arion species in Europe using a Bayesian analysis with а GTR + | + G model. Species and hybrid names are given based on genital morphology including ligula; green = A. vulgaris, blue = A. ater, red = A. rufus.

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The colour variation in A. vulgaris is remark-
able; red, brown, dark brown to almost black,
clearly overlapping with A. ater. However,
the brown morph of A. vulgaris was the most
abundant. Coloration seems to be only partially
associated with genital morphology in these large arionids, confirming the previously de-scribed uncertainty of identifying these species based on coloration alone (Cain & Williamson, 1958; Evans, 1986). However, coloration of juveniles makes them easier to differentiate (Quinteiro et al., 2005). Juvenile A. ater and A. rufus lack or have only faint lateral stripes which disappear when black and orange pig-ments develop (Brohmer et al., 1956; Kerney et al., 1979; Okland, 1922), while the distinct lateral bands of A. vulgaris are retained longer, sometimes until maturity (Dolmen & Winge, 1997; von Proschwitz, 1989; von Proschwitz & Winge, 1994). Interestingly, the topotype A. lusitanicus always retains these lateral bands to maturity (pers. obs.).
The variability of ligula morphology in Arion ater indicates introgression with A. rufus. The distinctive and invariable form of the A. ater ligula, as Noble described (1992), provides a reliable character to readily distinguish between A. ater and potential hybrids. We found the same variation in ligula as outlined by Noble (1992), with the addition of two quite distinct ligula forms (Fig. 2B and 2E), indicative of introgression with A. rufus. The same clear distinction was not found in the morphometric analyses (Figs. 4, 5) confirming previous stud-ies using morphometrics (Evans, 1986). Arion ater were mainly black (the colour variety var. atra), although very dark brown specimens (var. castanea — characteristic of A. ater x A. rufus hybrids) were also found. The var. atra colour variety is the commonest in northern Europe including Norway (@kland, 1922), and indicates a lack of introgression, except where var. castanea is also recorded, such as in the vicinity of Bergen in the 19th Century (Friele, 1853), where A. rufus was introduced. Here are no further records of A. rufus in Norway after 1890 (Okland, 1922), until the recent introduction recorded in 2008 in Selbu, central Norway (von Proschwitz & Andersen, 2010). It is therefore likely that A. rufus has introgressed with A. ater in Norway since the early nine-teenth century, leaving a residual mitochondrial signal which has spread to many populations. However, an equally likely explanation is that hybrids between A. ater and A. rufus have

been introduced to Norway, since a wide hybrid zone between these two species has been found in Denmark and northern Germany (Noble & Jones, 1996). Arecent introgression of A. ater, or A. ater-like hybrids, is supported by the finding that most of the individuals from southwest Norway (Klepp and Ha populations) displayed various combinations of ligula mor-phology, nDNA and mtDNA. If, as suspected (Noble & Jones, 1996), the success of such hybrids is a consequence of heterotic effects then the Klepp population may represent a recent introduction, or an area of extensive introgression. Such hybrid swarms are also common in the British Isles, where pure A. after populations have an increasingly restricted distribution (Noble & Jones, 1996). In such cases of continuing introgression, the genetic signature of less fecund taxa is assimilated (Selander & Hudson, 1976), leaving a residual mtDNA signal, or less common alleles from nuclear markers; these latter are eventually lost from the population by selection or drift. Alternatively, where introgression has been episodic, the result of multiple introductions of limited numbers of individuals, their greater fecundity may cause their genetic signature, in particular their mtDNA, to persist on the genetic background of the endemic taxon. As predicted we found many A. ater-like slugs with A. rufus mtDNA. A recent study by Rowson et al. (2014) found similar disagreements in taxonomic characters suggesting introgres-sion between large arionids in the British Isles. Studies involving more nuclear markers (e.g. microsatellites) may further elucidate these issues.
Arion vulgaris shows much less varia-tion in ligula morphology, conforming to the “Scandinavian taxon” proposed by Campbell (2000). Furthermore, in terms of mtDNA this taxon was genetically quite different from the Iberian taxa; A. /usitanicus, A. nobrei, and A. flagellus. This distinction was also evident from RAPD markers (Noble & Jones, 1996). In fact, Quinteiro et al. (2005) established that the non-topotype A. /usitanicus (A. vulgaris) outside the “Atlantic clade” (of A. lusitanicus (Mabille) and A. flagellus) is more related to A. ater and A. rufus than to the rest of the A. lusitanicus aggregate. These findings were confirmed by our COl-sequences, which clustered A. vulgaris with A. ater and A. rufus. However, RAPD markers (Noble & Jones, 1996) suggested close affinities between Aus-

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trian A. vulgaris and taxa from the Pyrenees, which Noble (1992) described as A. lusitanicus forms A and B. Intriguingly COl-sequences also suggest hybrids between A. vulgaris and A. ater, findings in accordance with studies in Sweden, suggesting hybrids based on genital morphology (von Proschwitz, 2009) and res-piration rates (Hagnell et al., 2003). Hybridiza-tion between A. vulgaris and A. ater may be consistent since hybrids between A. ater and A. rufus obscure the distinctiveness of these taxa, and potential mates may have problems identifying conspecifics. Thus hybrids may be a conduit for gene flow between species which would not otherwise mate. However, ecologi-cal differences between the species may limit introgression to hybrid zones. In particular, A. vulgaris and A. ater differ in habitat preference, where the former is found mainly in highly dis-turbed areas while the latter is also common in various natural or semi-natural habitats (e.g., moorlands, forests). Hence, hybrids may be predicted to occupy anthropogenic habitats (e.g. gardens, parks), as seems to be the case in our study.
The present work shows the importance of integrating several methods to distinguish between species in this complex group of facultatively self-fertile slugs. Based on these methods we conclude that A. vulgaris is quite distinct from A. lusitanicus, being more closely related to the A. ater aggregate. Furthermore, introgression between A. vulgaris and the A. ater aggregate seems to occur, possibly maxi-mizing the evolutionary potential and vigour of these similar genomes. However, the ability of many large arions to self-fertilize (Hagnell et al., 2006), producing founding colonies from a single progagule complicates the situation by adding more possibilities for relatedness be-tween generations of arionids. The taxonomic status of these slugs will probably remain controversial.

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LITERATURE CITED

ANDERSON, R., 2005, An annotated list of the non-marine Mollusca of Britain and Ireland. Journal of Conchology, 38: 607-637.
BARKER, G. M., 2002, Molluscs as crop pests. CABI Publishing, Wallingford, U.K., 400 pp. BROHMER, P., Р. EHRMANN & С. ULMER, 1956, Die Tierwelt Mitteleuropas, Band Il: Mollusca. Von Quelle, Leipzig, 264 pp.
BURNET, B., 1972, Enzyme protein polymorphism in the slug Arion ater. Genetic Research, 20: 161-173. |
CAIN, А. J. & M. WILLIAMSON, 1958, Variation and specific limits in the Arion ater aggregate. Proceedings of Malacological Society London, 33: 72-86.
CAMPBELL, Е. M., 2000, Investigating the inva-sive nature of the large arionid slugs of Europe: a study of recent invasions of Arion lusitanicus Mabille in Scandinavia. PhD thesis. Aberdeen University, Aberdeen, 46 pp.
CASTILLEJO, J., 1997, Babosas del Noroeste Iberico. Universidade de Santiago de Compost-ela, Santiago de Compostela, Spain, 192 pp. COLLINGE, W. E., 1893, On the occurrence of Arion lusitanicus Mab. in the British Isles, and descriptions of four new varieties. Annals and Magazine of Natural History, 12: 414-415. DAVIES, $. M., 1987, Arion flagellus Collinge and A. lusitanicus Mabille in the British Isles: a mor-phological, biological and taxonomical investiga-tion. Journal of Conchology, 32: 339-354. DOLMEN, D. & K. WINGE, 1997, Boasneglen (Limax maximus) og iberiasneglen (Arion lusitan-icus) iNorge; utbredelse, spredning og skadevir-kninger. “Limax maximus and Arion lusitanicus in Norway: distribution, expansion and injurious effects.” Report 4: 4-24. In: Zoological Series, Vitenskapsmuseet, Trondheim, Norway. DREIJERS, E:, Н. REISE & J. М. С. HUTCHIN-SON, 2013, Mating of the slugs Arion lusitanicus auct. non Mabille and A. rufus (L.): Different genitalia and mating behaviours are incomplete barriers to interspecific sperm exchange. Jour-nal of Molluscan Studies, 79: 51-63.
EVANS, N. J., 1986, An investigation of the status of the terrestrial slugs Arion ater ater (L.) and Arion ater rufus (L.) (Mollusca, Gastropoda, Pulmonata) in Britain. Zoologica Scripta, 15: 313-322.
FALKNER, G., R. A. BANK & T. von PROSCH-WITZ, 2001, Check-list of the non-marine molluscan species-group taxa of the states of Northern, Atlantic and Central Europe (CLE-COM Area I) and their distribution. Heldia, 4: 1-76.
FALKNER, G., Т.Е. J. RIPKEN & M. FALKNER, 2002, Mollusques continentaux de France liste de reference annotee et bibliographie. Museum d Histoire Naturelle, Patrimoines Naturels, 52: 350 pp.
FELSENSTEIN, J., 1985, Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39: 783-791.

918 HATTELAND ET AL.

FOLMER, O., M. BLACK, W. НОЕН, R. LUTZ &
R. VRIJENHOEK, 1994, DNA primers for ampli-
fication of mitochondrial cytochrome c oxidase
subunit | from diverse metazoan invertebrates.
Molecular Marine Biology and Biotechnology,
3: 294-299.
FOLTZ, D. W., H. OCHMAN, J. S. JONES, S. M. EVANGELISTI & R. K. SELANDER, 1932, Genetic population structure and breeding systems in arionid slugs (Mollusca: Pulmonata). Biological Journal of the Linnean Society, 17: 225-241.
FOLTZ, D. W., Н. ОСНМАМ & К. К. SELANDER, 1984, Genetic diversity and breeding systems in terrestrial slugs of the families Limacidae and Arionidae. Malacologia, 25: 593-605.
FRIELE, J., 1853, Norske land- og ferskvands-mollusker, som findes io megnen af Christiania og Bergen [in Norwegian]. “Norwegian terres-trial and limnic molluscs in the areas around Christiania and Bergen”. Feilberg & Landmark. Christiania, Norway, vi + 65 pp.
FREUND, К. J.,R.C. LITTELL & L. CREIGHTON, 2003, Regression using JMP software. 1st ed. SAS press, Cray, North Carolina, USA, 288 Pp.
GRIMM, B., W. PAILL & H. KAISER, 2000, Daily activities of the pest slug Arion lusitanicus. Jour-nal of Molluscan Studies, 66: 125-130. HAGNELL, J., C.S CHANDER & T. von PROSCH-WITZ, 2003, Hybridisation in arionids: the rise of a super slug? Pp. 221-226, in: С. Dussarr, ed., Slugs & snails: agricultural, veterinary & environmental perspectives. British Council For Crop Protection Symposium Proceedings, 324 pp.
HAGNELL, J., T. von PROSCHWITZ & C. SCHAN-DER, 2006, Self-fertilising observed in the invasive Iberian slug Arion lusitanicus Mabille 1868. Journal of Conchology, 39: 107.
HALL, T. A., 1999, BIOEDIT: a user-friendly bio-logical sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95-98.
HATTELAND, В. A., W. О. С. SYMONDSON, К. A. KING, М. SKAGE, С. SCHANDER & T. SOLH®Y, 2011, Molecular analysis of predation by carabid beetles (Carabidae) on the invasive Iberian slug Arion lusitanicus. Bulletin of Ento-mological Research, 101: 675-686. HATTELAND, B. A., S. ROTH, A. ANDERSEN, К. KAASA, В. STYA & T. SOLH®Y, 2013a, Distribution and spread of the invasive slug Arion vulgaris Moquin- Tandon in Norway. Fauna Norvegica, 32: 13-26.
HATTELAND, B. A., S. HAUKELAND, S. ROTH, М. В. BRURBERG, |. VAUGHAN & W. О. С. SYMONDSON, 2013, Spatiotemporal analysis of predation by carabid beetles (Carabidae) on nematode infected and uninfected slugs in the field. PLoS ONE, 8(12): eB2142. doi:10.1371/ journal.pone.0082142.
HILLEBRAND, Н. & В. MATTHIESSEN, 2009, Biodiversity in a complex world: consolidation and progress in functional biodiversity research. Ecology Letters, 12: 1405-1419.

HUELSENBECK, J. P. & Е. RONQUIST, 2001, MRBAYES: Bayesian inference of phylogeny. Bioinformatics, 19: 1572-1574.
KERNEY, М. Р., R. A. D. CAMERON & J. H. JUNGBLUTH, 1979, Die Landschnecken Nord-und Mitteleuropas. Paul Parey, Hamburg and Berlin, 384 pp.
KOZLOWSKI, J., 2007, The distribution, biology, population dynamics and harmfulness of Arion lusitanicus Mabille, 1868 (Gastropoda: Pul-monata: Arionidae) in Poland. Journal of Plant Protection Research, 47: 219-230.
LINNAEUS, C., 1758, Systema naturae, vol. 1. Stockholm, Salvii, 824 + iii pp.
MABILLE, M. J., 1868, Des limaciens européens. Revue et Magasin du Zoologie Pure et Appli-qu2ee0: 129,-14 6.
MOQUIN-TANDON, A., 1855, Histoire naturelle des mollusques terrestres et fluviatiles de France (Atlas), Paris, 92 pp., 54 pls.
NOBLE, L. R., 1987, The ecological genetics of reproduction in North-West European arionids. PhD thesis. University of London, London. NOBLE, L. R., 1992, Differentiation of large ari-onid slugs (Mollusca, Pulmonata) using ligula morphology. Zoologica Scripta, 21: 255-263. NOBLE, L. R. & J. S. JONES, 1996, A molecular and ecological investigation of the large arionid slugs of North-West Europe: the potential for new pest species. Pp. 93-131, in: W. O. C. SYMONDSON & J. E. LIDDELL, eds., The ecology of agricultural pests: biochemical approaches. Chapman & Hall, London.
NYLANDER, J. A. A., 2004, MrModeltest 2.2. Program distributed by the author. Evolutionary Centre, Uppsala University, Sweden.
OKLAND, F., 1922, The Arionidae of Norway. Videnskapsselskapets Skrifter Christiania, 1922: 1-62.
POLLONERA, A., 1889, Nuove contribuzioni allo studio degli Arion europei. Atti Reale Accademia della Scienze di Torino, 24: 623-640.
PORT, G. R. & A. ESTER, 2002, Gastropods as pests in vegetable and ornamental crops in Western Europe. Pp. 301-314, in: G. M. BARKER, ed., Molluscs as crop pests: CABI Publishing, Wallingford, U.K., 400 pp.
PORT, C. M. & G. R. PORT, 1986, The biology and behaviour of slugs in relation to crop dam-age and control. Agricultural Zoology Reviews, Intercept, Newcastle-upon-Tyne, 1: 255-299. QUICK, H. E., 1947, Arion ater (L.) and A. rufus (L.) in Britain and their specific differences. Journal of Conchology, 22: 249-261.
QUICK, H. E., 1960, British slugs (Pulmonata: Testacellidae, Arionidae, Limacidae). Bulletin of the British Museum (Natural History) Zoology, 6: 103-226.
QUINTEIRO, J., J. RODRIGUEZ-CASTRO, J. CASTILLEJO, J. IGLESIAS-PINEIRO & M. REY-MENDEZ, 2005, Phylogeny of slug species of the genus Arion: evidence of monophyly of Iberian endemics and of the existence of relict species in Pyrenean refuges. Journal of Zoo-logical Systematics & Evolutionary Research, 43: 139-148.

INVASIVE ARION SLUG 319

REISCHUTZ, P. L., 1984a, Zum massenhaften Auftreten von Arion lusitanicus Mabille in den Jahren 1982 und 1983. Mitteilungen aus dem Zoologischen Gesellschaft, Braunau, 4: 253-254.
REISCHUTZ, P. L., 1984b, Zur Schadwirking der „Kapuzinerschnecke“ Arion lusitanicus Mabille im Alpengebiet. Heldia, 1: 39.
RONQUIST, F. & J. P. HUELSENBECK, 2003, MRBAYES 3: Bayesian phylogenetic infer-ence under mixed models. Bioinformatics, 20: 407-415.
SELANDER КБ. К. & К. О. HUDSON, 1976, Animal population structure under close inbreeding: the land snail Rumina in Southern France. The American Naturalist, 110: 695-718.
SOROKA, М. 8 T. KALUSKI, 2011, Genetic stud-ies on the invasive slug Arion lusitanicus Mabille, 1868 (Gastropoda: Pulmonata) in Poland. Folia Malacologica, 19: 259-265.
ROTH, S., В.А. HATTELAND & Т. SOLH®Y, 2012, Some notes on reproductive biology and mat-ing behaviour of Arion vulgaris Moquin-Tandon 1855 in Norway including a mating experiment with a hybrid of Arion rufus (Linnaeus 1758) x A. ater (Linnaeus 1758). Journal of Conchology, 41: 249-257.
ROWSON, B., R. ANDERSON, J. A. TURNER & W. O. C. SYMONDSON, 2014, The slugs of Britian and Ireland: undetected and unde-scribed species increase a well-studied, eco-nomically important fauna by more than 20%. PLOSONE 9 (3): e91907. doi: 10.1371/journal. pone.0091907.
SOUTH, A., 1992, Terrestrial slugs — biology, ecology and control. Chapman & Hall, London, 428 pp.
SWOFFORD, D. L., 1998, Paup*. Phylogenetic analysis using parsimony (*and other meth-ods), version 4. Sunderland, M. A. Sinauer Associates.

VAN REGTEREN ALTENA, С. O., 1955, Notes sur les limaces. 3. Sur la presence en France d’ Arion lusitanicus Mabille. Journal de Conchy-liologie, 95: 89-99.
von PROSCHWITZ, T., 1989, Arion lusitanicus Mabille — en för Sverige ny snigelart. „Arion lusitanicus Mabille — a new slug species for Sweden“. Göteborgs Naturhistoriska Museum Arstryck, 1989: 43-53.
VON PROSCHWITZ, T., 1992, Den spanska skogsnigeln Arion lusitanicus Mabille - en art i snabb spridning med människan i Sverige. (with English summary: The Spanish slug Arion lusi-tanicus Mabille — an anthropochorous slug spe-cies spreading rapidly in Sweden). Göteborgs Naturhistoriska Museum, 1992: 35-42.
von PROSCHWITZ, T., 1997, Arion lusitanicus Mabille and A. rufus (L.) in Sweden: a compari-son of occurrence, spread and naturalization of two alien slug species. Heldia, 4: 137-138.
von PROSCHWITZ, T., 2009, Faunistical news from the Natural History Muesum, Göteborg 2008 — snails, slugs and mussels — with some notes on the slug Limacus flavus (Linnaeus) — refound in Sweden, and Balea heydeni von Maltzan — a land snail species new to Sweden. Göteborgs Naturhistoriska Museum Arstryck, 2009: 47-68.
von PROSCHWITZ, T. & A. ANDERSEN, 2010, Radskogsnegl Arion rufus i Sverige og Norge (In Norwegian with English Summary). “Red slug Arion rufus in Sweden and Norway”. Fauna, 63: 2-7.
von PROSCHWITZ, Т. & К. WINGE, 1994, Бе-riaskogsnegl — en art pá spredning i Norge. “The Iberian slug — a species expanding in Norway.” Fauna, 47: 195-203.

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